

VIRGINIA RECREATIONAL FISHING DEVELOPMENT FUND SUMMARY PROJECT APPLICATION*

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NAME AND ADDRESS OF APPLICANT: Virginia Institute of Marine Science PO Box 1346 Gloucester Point, VA 23062	PROJECT LEADER (name, phone, e-mail): Dr. Mary C. Fabrizio Phone: 804-684-7308 Email: mfabrizio@vims.edu			
PRIORITY AREA OF CONCERN: Research	PROJECT LOCATION: Virginia Institute of Marine Science			
DESCRIPTIVE TITLE OF PROJECT: Response of summer flounder to hypoxia in Chesapeake Bay: physiological tolerances and shifts in habitat use				
PROJECT SUMMARY: During the summer and early fall, summer flounder use shallow coastal areas such as the Chesapeake Bay and eastern shore inlets and bays for rapid growth. Recent increases in the geographic extent and volume of hypoxic waters may negatively affect the availability of suitable habitats for these fish. We propose to combine laboratory and field studies to understand the effect of hypoxia on habitat use by summer flounder and the ability of the fish's cardio-respiratory system to maintain oxygen delivery during acute hypoxia.				
EXPECTED BENEFITS: Our combined field and laboratory results will allow us to understand how the physiological responses of summer flounder affect their habitat use and movement patterns relative to changing environmental conditions. Additionally, the laboratory experiments will provide important information on how areas of low oxygen concentration can influence summer flounder growth, metabolism, and survival. Results of this study will provide information on the use of hypoxic areas by summer flounder and allow the development of guidelines to assist anglers in avoiding capture of stressed fish. This may be particularly important if the laboratory portion of the project shows that fish from hypoxic areas are less tolerant of the stresses associated capture-and-release.				
COSTS: <div style="display: flex; justify-content: space-between; align-items: flex-start; margin-top: 20px;"> <div style="width: 30%;"> VMRC Funding: Recipient Funding: Total Costs: </div> <div style="width: 60%;"> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="text-align: right; padding: 5px;">\$ 99,721</td> </tr> <tr> <td style="text-align: right; padding: 5px;">\$ 38,178</td> </tr> <tr> <td style="text-align: right; padding: 5px;">\$ 137,899</td> </tr> </table> </div> </div>		\$ 99,721	\$ 38,178	\$ 137,899
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Detailed budget must be included with proposal.				

Updated 6/1/05

*This form alone does not constitute a complete application, see application instructions or contact Sonya Davis at 757-247-8155 or sonya.davis@mrc.virginia.gov : Due dates are June 15 (Jul. – Nov. Cycle) and December 15 (Jan. – May Cycle)

Proposal Submission to
VMRC RECREATIONAL FISHING ADVISORY BOARD

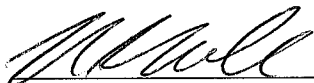
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THE VIRGINIA INSTITUTE OF MARINE SCIENCE
COLLEGE OF WILLIAM AND MARY

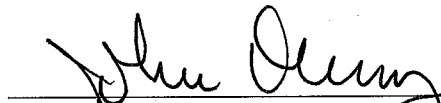
RESPONSE OF SUMMER FLOUNDER TO HYPOXIA IN CHESAPEAKE BAY:
PHYSIOLOGICAL TOLERANCES AND SHIFTS IN HABITAT USE




Dr. Mary C. Fabrizio
Principal Investigator



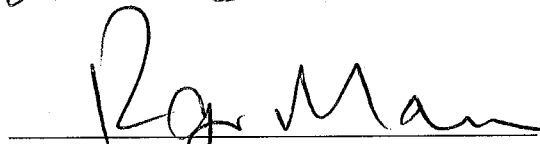
Dr. Richard W. Brill
Co-Principal Investigator



Dr. John Olney
Chair, Department of Fisheries Science



Ms. Jane A. Lopez
Director, Sponsored Programs



Dr. Roger Mann
Director for Research and Advisory Services

December 2006

December 2006

Response of summer flounder to hypoxia in Chesapeake Bay: physiological tolerances and shifts in habitat use

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Need:

During the summer and early fall, summer flounder (*Paralichthys dentatus*) use shallow coastal areas such as the Chesapeake Bay and eastern shore inlets and bays for rapid growth. Recent increases in the geographic extent and volume of hypoxic waters in the Bay may negatively affect the availability of shallow habitats for these fish. Summer flounder may avoid hypoxic waters if they can detect decreasing concentrations of dissolved oxygen, or they may be trapped in hypoxic waters because other factors such as temperature or depth constrain their movements. Although surveys combined with environmental monitoring can provide information on the occurrence of fish relative to environmental factors such as dissolved oxygen, they provide only a 'snap shot' of the population and yield little insight into the mechanisms contributing to the observed distributions. For example, trawl surveys of summer flounder in a tributary of Delaware Bay provided ambiguous information on the movement of fish in response to hypoxia (Tyler 2005). Furthermore, laboratory experiments indicate that summer flounder avoided 1 mg O₂ L⁻¹ waters, but remained sedentary when dissolved oxygen levels were 2 mg O₂ L⁻¹ indicating that avoidance of hypoxic waters (<2 mg O₂ L⁻¹) may not be initiated soon enough to avoid compromising or lethal conditions (Tyler 2005). Dissolved oxygen concentrations of 3.5 mg O₂ L⁻¹ or less can reduce growth rates of juvenile summer flounder (Stierhoff et al. 2006). Such laboratory experiments suggest that summer flounder may not be capable of detecting oxygen concentrations that are low enough to compromise their growth and metabolism.

Background:

Field studies

Summer flounder is one of the most highly prized sport fishes of the eastern US seaboard. They occur in marine and estuarine waters from Nova Scotia to South Carolina, and support fisheries from Massachusetts to North Carolina (Bigelow and Schroeder 1953; Terceiro 2001). In Virginia, 659,618 kg of summer flounder were harvested by saltwater anglers in 2005 (pers. comm., National Marine Fisheries Service, Fisheries Statistics Division). Summer flounder are harvested by the recreational fishery during spring, summer, and fall when fish inhabit estuaries and shallow coastal waters, and by the commercial fishery during winter months when fish occupy the deeper waters of the continental shelf. Although the distribution, abundance, and status of the offshore population during winter is assessed annually with seasonal bottom trawl surveys conducted by NOAA-Fisheries Service, Northeast Fisheries Science Center, relatively

little is known about summer flounder distribution and movements in inshore waters. For example, most of the adult summer flounder captured by bottom trawls in the mainstem of the Chesapeake Bay in recent years appear to be female fish (R. Latour and C. Bonzek, pers. comm., VIMS). According to recent results from the Virginia Game Fish Tagging Program, fish less than the minimum size (16.5 inches or 419 mm total length [TL]) may exhibit some degree of site fidelity during the period of estuarine use (Lucy and Bain 2005). Thus, size and sex may be important factors contributing to variation in summer flounder estuarine distribution and habitat use in Virginia coastal waters. Growing concerns over persistent and expanding areas of low-oxygen bottom waters during summer underscore the need to understand the distribution of summer flounder in the Virginia's coastal waters. We propose to use state-of-the-art tagging technology to study habitat associations and localized movements in response to hypoxia of individual summer flounder.

Trawl surveys provide a wealth of information on the distribution and the seasonal migration patterns of summer flounder. In the spring, adult summer flounder move inshore to inhabit coastal and estuarine waters. In the fall, fish migrate offshore towards the continental shelf break to spawn off the coast of New Jersey, Virginia, North Carolina, or south of Cape Hatteras (Kraus and Musick 2001). Larval summer flounder enter estuarine waters of Chesapeake Bay beginning in October but as late as May (Norcross and Wyanski 1994; Murdy et al. 1997). Young-of-the-year summer flounder within the Chesapeake Bay are vulnerable to bottom trawls as early as March and remain in the system until December; however, the period of peak recruitment to the gear is September through November (Montane and Lowery 2005).

To further understand the movement patterns of summer flounder some studies have used mark-recapture techniques. Mark-recapture (or tagging) studies are commonly used in fisheries to understand fish movement as well as to estimate population parameters such as survival and emigration. For a tagging study to be successful, sufficient numbers of fish must be tagged to provide a good indication of the population-level processes affecting the numbers, movement, and distribution of individuals. Additionally, tagging studies rely on the reporting of any recaptures that occur and the fate of the fish that were recaptured (e.g. released alive or harvested). Results from previous mark-recapture studies on summer flounder have provided some indication of their movement patterns, but are difficult to interpret due to low recapture rates or because studies were not designed to examine specific ecological questions. Kraus and Musick (2001) used mark-recapture data from 10,607 juvenile summer flounder (<290 mm TL) tagged and released in Chesapeake Bay and Virginia coastal waters to examine the question of stock structure; most of the fish recaptured after 40 days at large moved north and were recaptured in coastal states from Maryland to Connecticut. However, these observations are based on extremely low recapture rates (0.2%) and may not reflect the movement of fish tagged from parts of the bay not studied (e.g., structured sites; Lucy and Bain 2005). Recaptures of summer flounder tagged as 229 - 381 mm [9-15 inch] indicate that small summer flounder may exhibit some site attachment or perhaps have small home ranges during their residency within the bay (Lucy and Bain 2005). In general, fish remained closely associated with structures (e.g., Hampton Roads Bridge Tunnel, Buckroe Pier, Gloucester Point Pier, and Yorktown Beach Jetties) or highly productive areas (e.g. Rudee Inlet) preferred for feeding and refuge. (Lucy and Bain

2005). In addition, the length of time young fish remain in Chesapeake Bay habitats (up to 150 days; Lucy and Bain 2005) may exceed that previously reported for New Jersey salt-marsh creeks, which was 17 days on average (Rountree and Able 1992). Although these studies have provided some information about summer flounder habitat use and movement patterns, they provide little information regarding the fine-scale movement patterns of these fish in response to dynamic processes, such as hypoxia.

In recent years, fish movements, home ranges, dispersal rates, and habitat use have been studied with ultrasonic telemetry (e.g., Hooze and Taggart 1998; Arendt et al. 2001; Cote et al. 2003; Parsons et al. 2003; Lowe et al. 2003; Heupel et al. 2004). This technology is similar to acoustic tracking technology used in wildlife studies, but uses acoustic signals in the ultrasonic range (generally 30-80 kHz) because higher frequency signals (e.g., radio waves) are absorbed rapidly in seawater (Pincock and Voegeli 2002). To our knowledge only three studies of summer flounder have been conducted to date with this technology. The first was applied to young-of-the-year fish (210-254 mm TL) in a New Jersey marsh creek but used only 9 fish (Szedlmayer and Able 1993). Another study occurred in the same area and was reported in December 2004 at the Flatfish Biology Conference¹. This work included 70 fish ranging in size from 267 to 533 mm and involved both active and passive tracking of fish in and around Great Bay-Little Egg Harbor, New Jersey. These fish were reported to have undertaken limited movements in the estuary from April through November. The third study involved 24 summer flounder >265 mm TL passively monitored off the coast of New Jersey using an acoustic grid (Fabrizio et al. 2005). Results from the study by Fabrizio et al. (2005) provided data which can be used to quantitatively estimate habitat use by summer flounder (Fabrizio et al., ms. in prep). Specifically, Fabrizio et al. (ms. in prep) are investigating the association between summer flounder occurrence and habitat features such as bottom slope, sediment type, and depth. Unlike previous studies, which describe summer flounder habitat associations using trawl survey data (e.g. Kraus 1998, Tyler 2005), this study is free from common sampling biases associated with trawl data such as gear (e.g., unknown catchability, efficiency), time of year (e.g., sampling restricted to a few days or weeks, at best), habitat type (e.g., sampling confined to trawlable sites), time of day (e.g., sampling during the day but not at night), and temporal intensity of sampling.

The properties of the study site must be considered when designing an acoustic telemetry study to passively monitor fish movement (Heupel et al. 2006). For instance, acoustic gates or “curtains” consisting of monitoring receivers positioned perpendicular to the direction of fish movement may be used in areas that are relatively narrow or otherwise confined by land on two or more sides, such as streams and rivers. In other cases, a study site may be encircled by receivers; this type of design is suitable for studies of some marine protected areas. Other habitats require the use of an acoustic grid or a more complex arrangement of monitoring receivers that permits detection of acoustic signals within study sites of various shapes and within portions of study sites (e.g., among two or more bottom habitat types). Previous telemetry studies examining the

¹K. Able, D. Rowles, and T. Grothues (Rutgers University Marine Field Station). An evaluation of summer flounder estuarine habitat use using acoustic telemetry. Ninth Flatfish Biology Conference, Westbury, CT, December 2004.

habitat use of summer flounder (Fabrizio et al. 2005) have used a grid design and this approach should also be the most appropriate to monitor fish movement patterns in relation to dynamic environmental changes (e.g. hypoxia).

Our proposed study will elucidate the movements of summer flounder in Virginia estuaries (where abundance of summer flounder is high) and examine movements and habitat use by fish in response to naturally occurring hypoxic events. Summer flounder will be implanted surgically with individually coded transmitters which will allow us to monitor the movements and habitat use of individual fish using a passive acoustic grid in an area prone to hypoxic events. In addition, we propose to examine the hypoxia tolerance of summer flounder by conducting a series of laboratory studies aimed at quantifying the ability of summer flounder to both sense hypoxia and to maintain rates of oxygen delivery under these conditions. Such experiments will involve measuring changes in heart rate, ventilation frequency, ventilation volume, gill oxygen extraction, and metabolic rate in sedentary fish during acute exposure to hypoxia.

Laboratory studies

Although recent results indicate that summer flounder growth rates decline during periods of hypoxia (Stierhoff et al. 2006), the metabolic response and the ability of the summer flounder cardio-respiratory system to maintain oxygen delivery during acute hypoxia is completely unstudied. As a result, the influence of hypoxia on the movements, distribution, ecology and energetics of this species are not understood. Hypoxia tolerance, moreover, varies widely among the teleosts. Some species are “oxygen regulators” and maintain oxygen delivery until the so-called “critical oxygen tension” is reached, after which metabolic rates fall in proportion to further decreases in ambient oxygen. Other species are “oxygen conformers,” in that metabolic rate decreases immediately with reductions in ambient oxygen. The order Pleuronectiformes (flatfishes) exemplifies this diversity of responses as some species in the order are oxygen conformers while others are oxygen regulators. For example, the eastern Atlantic flounder (*Platichthys flesus*) and Pacific starry flounder (*P. stellatus*) are both relatively hypoxia tolerant and maintain nearly constant oxygen uptake rates down to ambient oxygen levels of $\approx 40\%$ saturation (Watters and Smith 1973; Steffensen et al. 1982). In contrast, plaice (*Pleuronectes platessa*) are oxygen conformers (Steffensen et al. 1982), and their metabolic rates fall immediately in direct proportion to decreases in ambient oxygen. For flounder and plaice, moreover, the differences in the ability of their cardio-respiratory systems to maintain oxygen delivery in the face of acute hypoxia are reflective of their respective distributions within eastern Atlantic coastal environments. Plaice generally occupy waters at depths of 10 to 50 m where environmental conditions tend to remain relatively stable; whereas eastern Atlantic flounder are common in shallower coastal environments where diurnal fluctuations in temperature, salinity and oxygen levels can be extreme (Muus 1967; Steffensen et al. 1982).

Objectives:

The objective of this study is to examine the effects of decreasing oxygen concentrations (hypoxia) on summer flounder by conducting laboratory experiments with captive fish and relating these observations to field-based telemetry information on habitat use. Specifically, we propose to:

- 1) quantify the physiological responses of summer flounder to hypoxia, including their ability to detect it as well as compensate for it both physiologically and behaviorally, and
- 2) determine patterns of habitat use of summer flounder in the presence of hypoxia.

Together, these studies will help us understand how summer flounder interact with their environment and how habitat use is altered in the presence of hypoxia. These results will have implications for management of the population (e.g., if growth is affected) and for delineating essential fish habitat for this species. In addition, the response of summer flounder to hypoxia will provide insights on the potential displacement of summer flounder to habitats that may be less suitable for growth or survival. Our work contributes directly to an understanding of the interaction of an exploited species (summer flounder) and its habitat and environmental stressors. This type of knowledge is the basis for the development of an ecosystem-based fisheries management plan for Chesapeake Bay (FEP 2004).

Expected Benefits:

This study will benefit the recreational fishery in Virginia by providing anglers and fishery managers with a better understanding of summer flounder habitat use and movement patterns relative to changing environmental conditions. Additionally, the laboratory experiments will provide important information on how areas of low oxygen concentration can influence summer flounder growth, metabolism, and survival. With the growing concern over low oxygen bottom waters during summer months, results from this study will provide an indication of how changes in oxygen concentration may influence summer flounder movement patterns and growth. These results will have implications for management of the population (e.g., if growth is affected) and for delineating essential fish habitat for this species.

We fully expect our results to provide a clear picture of the ability of summer flounder to function under various levels of hypoxia. For example, we will know to what level of hypoxia flounder are able to maintain oxygen delivery to the tissues, and at what level they must begin to rely on anaerobic metabolism. Flounder will not be able to remain indefinitely below this threshold. From the onset of cardio-respiratory responses, we also get an estimate of the minimal decreases in ambient oxygen levels summer flounder are able to detect. In brief, the laboratory results will allow the behavioral responses of flounder recorded during the field study to be interpreted unequivocally, and stronger prediction to be made as to the relationship between hypoxia and suitability of flounder habitat. Field data from telemetered summer flounder will provide information on the effects of various environmental factors (e.g., tidal stage, temperature and dissolved oxygen) on summer flounder movements and habitat use. This body of work will contribute to an understanding of environmental stressors present in the areas identified as essential fish habitat for summer flounder (Chesapeake Bay and eastern shore seaside inlets and bays).

Approach:

This project consists of both a field component and a laboratory component. Beginning in June 2007 we will conduct a field study with telemetered summer flounder in the estuaries behind the barrier islands on Virginia's eastern shore. We have chosen to

conduct our experiments here because relative to the Chesapeake Bay, these are geographically smaller areas, which can be more effectively monitored with fewer ultrasonic receivers and environmental monitors. Moreover, these areas are routinely prone to acute hypoxic events (R. Brill, personal observation). After the completion of field work we will conduct laboratory experiments to determine how well summer flounder tolerate acute reductions in ambient oxygen levels. Below, we describe in detail the methods for the two types of experiments.

Field study: deployment of receivers and data sondes

We will use ultrasonic telemetry to continuously monitor the habitat use of summer flounder in an eastern shore estuary. Specifically, passive acoustic arrays will be deployed in tidal creeks and a small bay near Wachapreague, VA. These tidal creeks are an ideal experimental unit because (1) they support high numbers of summer flounder; (2) they are relatively small and thus tractable with a modest amount of acoustic equipment; and (3) they are susceptible to hypoxia during periods of low tide. The hypoxic events in the tidal creeks are thought to be related to the high rates of algal respiration in shallow portions of the creek during night. In addition to the acoustic receivers we will deploy one or more data sondes to record temperature, salinity and dissolved oxygen concentrations at the study site. The combination of acoustic arrays and data sondes will permit tracking of individual fish throughout the course of various cycles thought to influence fish movement (i.e, tidal cycles and the cyclical changes in dissolved oxygen concentrations).

To prepare for the deployment of receivers, we will conduct a range test to determine the maximum distance at which a transmitter can be detected in our study site. This test provides site-specific information on the likelihood of signal detection by a receiver as a function of distance between the transmitter (emitting the signal) and the receiver (detecting and decoding the signal). A range test is necessary for optimal placement of receivers because receiver detection distances vary widely as a function of environmental characteristics (e.g. water depth, turbidity, and the presence of soniferous organisms; Pincock and Voegeli 2002). Based on a preliminary test with similar gear in a shallow, vegetated area (Lucy and Machen 2003), we estimate the detection distance to be at least 200 and up to 300 m. Detection ranges in non-vegetated waters are expected to be greater than 300 m. The range test will be conducted from a small vessel using a single moored receiver and a transmitter that will be placed at known distances to the receiver.

When deployed, each receiver will be attached to a mooring and a surface float to mark its location. In addition to the surface float, the location of each receiver will be logged according to its GPS position. The receiver-mooring array will also be equipped with temperature data loggers to record temperature at the study sites. We will also deploy a YSI extended deployment system data sonde with capability to measure and record dissolved oxygen concentrations. The data sonde will be moored in a similar fashion in the study area. Each array (receivers, data sonde) will be routinely monitored and cleaned of fouling organisms. Receivers passively detect, decipher, and record transmissions from ultrasonic transmitters; the information (date, time of day, transmitter identification number) is stored in the memory of the receiver. To obtain these data, the

receiver and temperature data logger must be retrieved (see below) and interfaced to a personal computer.

Field study: Release of summer flounder with surgically implanted transmitters

In early summer (June) we intend to capture sixty summer flounder using hook and line or by trawling, and surgically implant the fish with individually coded transmitters. The implantation procedure for summer flounder has been developed and is described in Fabrizio et al. (2005). Briefly, summer flounder are anesthetized, a small incision is made on the non-ocular side (non-pigmented side), a beeswax-coated transmitter is inserted into the peritoneal cavity, and the incision is stitched using non-absorbable sutures in an interrupted pattern. While the fish is anesthetized, size and weight measurements are collected, and an individually numbered anchor tag is inserted into the dorsal musculature. Fish are then resuscitated using ram ventilation and released. In addition to providing external identification of the fish, the anchor tag will have a phone number to call should the fish be recaptured by anglers or commercial fishers. All fish will be captured at the study sites and released at location of capture. The timing of the field work (early to late June) will depend on when fish >265 mm TL are available to our gear (either trawl or hook and line). Summer flounder smaller than 265 mm TL should not be implanted with 30 mm transmitters; incisions through the thin tissues of small fish are difficult to do without rupturing internal organs, and mortality is high with fish of this size (Fabrizio et al. 2005).

We will use coded transmitters (i.e., transmitters that transmit individual codes and hence, identify individual fish); these transmitters will be configured to ensure battery power for the duration of the study (i.e., the duration of estuarine habitat use for summer flounder, about 8-9 months). Battery life is a function of the size of the transmitter, type of battery, and the length of the delay between coded transmissions (Pincock and Voegeli 2002). Excellent results were obtained by Fabrizio et al. (2005) using coded transmitters 30 mm long and 9 mm in diameter with a delay time varying between 180 and 300 seconds. With this configuration, battery life was about one year. We propose to shorten the delay time to obtain a battery life about 8 months, thus allowing tracking of individual fish from June through January.

Field study: Retrieval and analysis of acoustic data

Acoustic receivers must be retrieved from the study site to permit acquisition of acoustic data; such retrievals will be conducted periodically. Once summer flounder leave the study site (we expect this to be in around December), we will retrieve the receivers and data sondes for a final time and download the data. Arrays will be removed from the study site at the completion of the field work in January, conditions permitting.

Field Study: Statistical approach

Following retrieval, we will analyze the acoustic data for information on habitat use and movements; site fidelity and dispersal from sites will also be examined. Data from the temperature loggers and dissolved oxygen data sonde will be downloaded as well. The influence of dissolved oxygen concentration, tidal stage, and water temperature will be explored relative to summer flounder movement and habitat use. We propose to examine habitat use with a negative binomial model; in this approach, the

number of detections at a particular receiver (the response variable) is assumed to follow a negative binomial distribution. This is a reasonable assumption because we anticipate the occurrence of zeroes in the detection data (that is, at certain times, there will be locations that are not occupied by our study fish) and because we expect the variance to be much larger than the mean. Based on our previous experience with detection data from monitoring receivers, other distributions, such as the Poisson, result in severe overdispersion, a condition that leads to underestimation of standard errors of model parameters and affects inference (e.g., Pedan 2001; Littell et al. 2002). The number of detections for a given receiver will be modeled as a function of depth, dissolved oxygen, temperature, distance from shore, bottom type, habitat type, and sediment characteristics using a generalized linear model. This type of model does not require the assumption of normally distributed data nor does it require the assumption of a symmetric distribution of errors (Littell et al. 2002). Thus, the generalized linear model is a more flexible approach, allowing the investigator to stipulate the distribution of the response variable, and the nature of the relationship between the mean response and the linear predictors (through the use of a link function such as the logit; Littell et al. 2002). We will use SAS to test for goodness-of-fit of the model and to obtain the maximum likelihood estimates of parameters. Movements of summer flounder from the study sites will be examined relative to tidal stage, temperature fluctuations, and dissolved oxygen with a repeated measures model. Because monitoring receivers record data throughout the day, observations on a given fish are serially correlated and are thus, repeated measures. We will develop an index to account for movement of fish within and among portions of our study site and use a repeated measures ANOVA to test for equality of movement through various time periods (days, weeks, months) and across environmental changes (temperature, dissolved oxygen, tide stage). Site fidelity can be examined using simple descriptive statistics that characterize length of time within a given study site. Dispersal rates, which represent movement of fish away from the site, can be estimated using the Kaplan-Meier (KM) approach (Bennetts et al. 2001). The KM method is a nonparametric approach, requiring no assumptions about the underlying hazard function. KM estimators are robust, have well described variances (Pollock et al. 1989a), and can be modified to permit staggered entry of individuals (Pollock et al. 1989b). We will use SAS to obtain maximum likelihood estimates of dispersal rates from the KM model (see Fabrizio et al. 2005). Finally, we will examine results from this study and compare summer flounder habitat use and dispersal to results from Fabrizio et al. (in prep). Analysis of the data and preparation of the final report for this study will require about 8 months time; we anticipate completion of the report by Sept 2008.

Laboratory studies

The overall objective for our laboratory studies will be to determine how well summer flounder tolerate acute reductions in ambient oxygen. Experiments will center on the cardio-respiratory responses of fish to decreasing oxygen levels, and will be designed to quantitatively describe summer flounder responses and ability to maintain rates of oxygen delivery under these conditions (i.e., define critical oxygen tension). Because of their laterally compressed body morphology and strong tendency to remain motionless and partially buried in bottom sediments for long periods, flatfishes are especially suitable for these types of laboratory experiments.

We will use standard physiological laboratory procedures to measure changes in heart rate, ventilation frequency, ventilation volume, gill oxygen extraction, and metabolic rate occurring in sedentary fish during hypoxia. These requisite procedures are described in detail by Watters and Smith (1973), Cech and Rowell (1976), Cech et al. (1977), Wood et al. (1979), Kerstens et al. (1979), and Steffensen et al. (1982). In brief, fish will be outfitted with indwelling arterial and venous catheters and rubber masks covering both opercular openings. These will allow sampling of both arterial and venous blood and exhaled water with no disturbance to the fish. The total volume of exhaled water will also be moved through a fixed diameter tube surrounded by an electromagnetic flow meter to measure ventilation volume. Attempts will be made to monitor cardiac output with pulsed Doppler electronic blood flow meter. If this proves not to be feasible, cardiac output will be calculated based on the Fick principle with corrections made for gill metabolic rate (Johansen and Pettersson 1981, Metcalf and Butler 1982; Bushnell and Brill 1992). Once the fish are fully instrumented, they will be moved to the experimental chamber, allowed to bury in a sandy substrate, and given approximately 48 hours to recover. Experiments will be run over several days, and will consist of measurements being made during normoxia and during three to five discrete stepwise reductions in ambient oxygen (down to approximately 20% air saturation). Ambient oxygen levels will be controlled by passing water through a gas exchange column (medical grade membrane oxygenator), and employing a custom designed computer-controlled gas mixing system similar to that described by Wood et al. (1975). Water will also be continuously circulated through an activated carbon filter and UV sterilizer to remove nitrogenous wastes and to prevent growth of micro-organisms.

Oxygen and CO₂ levels in the blood, as well as plasma pH, will be measured with temperature-controlled electrodes and Radiometer blood gas analyzers, and blood oxygen content will be measured with a standard Tucker chamber. Oxygen levels in inspired and expired water will likewise be measured with the same Radiometer system. From these data, plus those on cardiac output and ventilation volume, the standard suite of parameters completely characterizing cardio-respiratory function during normoxia and various levels of hypoxia will be calculated as described by Bushnell and Brill (1992).

Laboratory studies – statistical analysis

Measured and calculated parameters describing cardio-respiratory function during normoxia and the various levels of hypoxia will be analyzed using standard ANOVA procedures as described by Bushnell and Brill (1992).

Location:

We propose to examine summer flounder habitat use and movement in response to hypoxia at tidal creeks near Wachapreague, Virginia. These creeks have been known to exhibit periods of hypoxia, especially during low tides. Although other areas of Chesapeake Bay routinely exhibit hypoxia or anoxia, the reliability of the hypoxic events is not known and summer flounder may not occur in high enough abundance at those sites. Thus, to maximize our chances at success, we propose to study a creek with known hypoxic events and with a large population of summer flounder. The Wachapreague area supports a healthy recreational fishery for summer flounder, thus ensuring our chances of capturing sufficient numbers of fish that will large enough to implant with acoustic

transmitters. We consider this study site as an excellent model of shallow water habitats throughout Chesapeake Bay, and thus, our results will be applicable to summer flounder populations in the Bay.

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Estimated Cost: \$ 99,534

Personnel - \$ 24,210

We are requesting salary support for 6% time for M. Fabrizio (6% matched by VIMS) and the yearly stipend for one graduate student. The salary for M. Fabrizio is supplemented by the customary 30% fringe benefit rate. R. Brill's contribution (10% time) is matched by the NOAA-Cooperative Marine Education and Research program.

Supplies - \$ 35,700

Supplies requested for this project include acoustic transmitters (for 60 fish), field supplies (e.g., anesthetic, sutures, tissue adhesive, gauze pads, surgeon's gloves, surgical instruments, waterproof paper, external T-bar tags, bait, epoxy, buckets, tools, electronics cleaner, coolers, measuring boards, etc., batteries for acoustic receivers, and array hardware costs (estimated at \$450 per array; we propose to construct 26 arrays). Arrays

include anchors, mooring buoys, high-strength line (such as Spectra or Amsteel), galvanized shackles, swivels, thimbles, cable ties, etc. We are also requesting funds for vessel fuel, which we estimate to be \$2,800, based on current VIMS fuel rates for vessels. We will be using existing holding tanks for this work.

Travel - \$ 5,000

Travel cost is estimated for trips to and from Wachapreague, VA. The estimated cost of vehicle rental was calculated based on current VIMS vehicle rental rates. Additionally, we included the cost of dorm rental at the VIMS eastern shore laboratory and a per diem to cover food costs. This is customary for VIMS researchers/students engaged in research at the eastern shore laboratory.

Equipment - \$ 3,300

We propose to purchase 3 “replacement” acoustic receivers suitable for mooring in the marine environment for extended periods of time. These 3 receivers will be supplemented by receivers already on hand (purchased previously with VMRC funds) for a total of 26 listening stations (26 arrays) at Wachapreague. We are requesting 3 receivers at this time because we are unsure how many we will be able to retrieve from the current deployment (that is, it is possible that some receivers will be lost), and because we cannot be certain that the recovered receivers will be in good working condition. Thus, to ensure we have adequate numbers of receivers, we are seeking funds for 3 “replacement” receivers. Based on previous experience with these systems, it is customary to need to replace about 10% of the units. Also, because acoustic receivers are specialized scientific research instruments, there are no sources for rental. Two YSI data sondes (estimated cost: \$13,200) will be purchased by the PI using other funding sources; these units will be used in this study to continuously monitor and record dissolved oxygen concentrations at the study site. We will also be deploying temperature sensors on the arrays using existing equipment (HOBO tidbits). An oxygen analyzer and regulator to control the ambient oxygen levels during laboratory experiments will also be purchased by the PI using other funding sources (estimated cost: \$2,100).

Vessel Rental - \$ 3,500

Vessel rental rates were calculated based on the VIMS eastern shore laboratory daily rate for a large Carolina skiff (27-foot vessel). Vessels will be used for the range test, collecting fish, and for deployment, maintenance, and retrieval of receivers and data sondes.

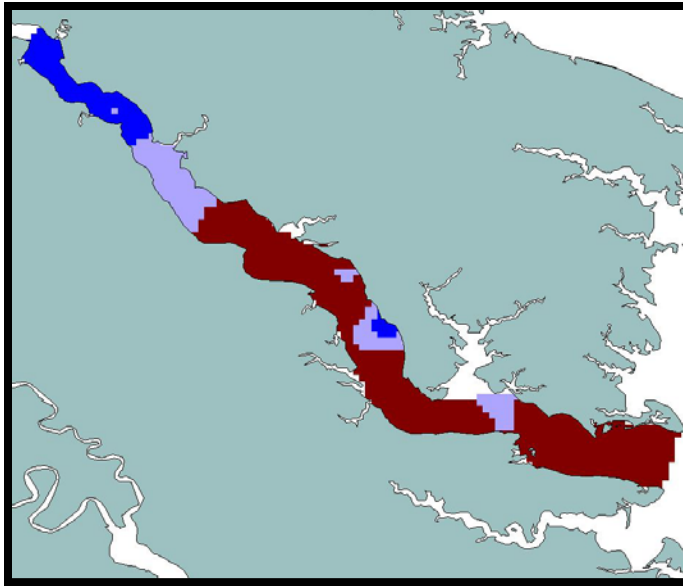
Subcontract - \$9,427

We are requesting funds to subcontract with Dr. P. Bushnell (Indiana University) who will perform laboratory analyses on the effects of reduced oxygen concentrations on the physiological response of summer flounder. This work is of a highly technical nature and requires the skills of an experienced physiologist. Dr. Bushnell has performed these experiments and is an expert on the topic. Training a graduate student to perform this work is not cost-effective and would significantly increase the amount of time (and money) necessary to complete the project.

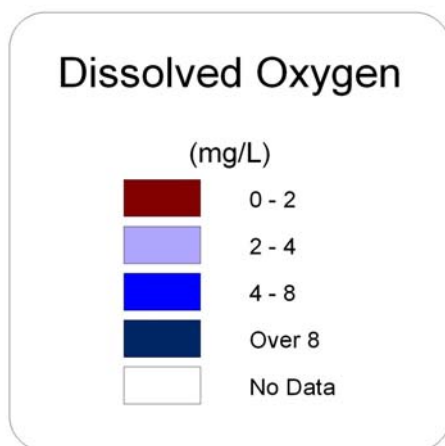
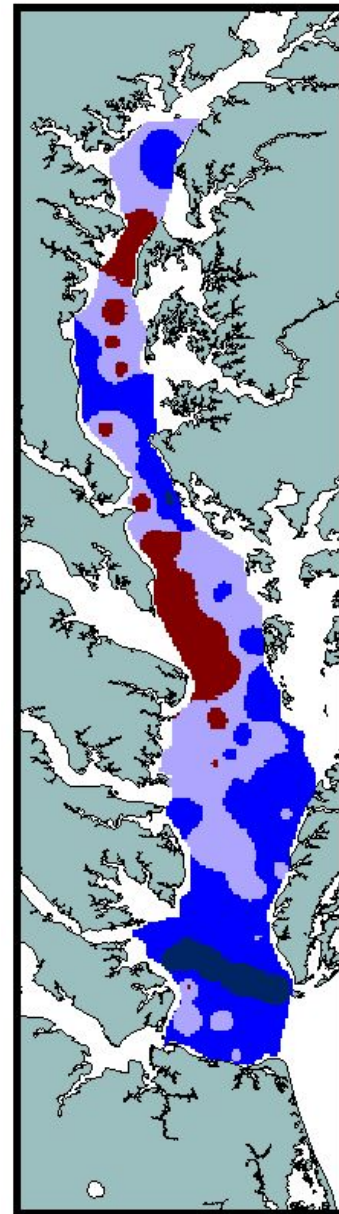
Indirect costs - \$ 36,584 (RFAB: \$ 18,547; VIMS match: \$ 18,037)
Facilities and administrative costs are calculated at 25% for funds provided by VMRC.
The VIMS indirect cost rate is 45%; the remaining indirect costs are contributed as part
of VIMS match for this project.

Figure 1. Dissolved oxygen concentration (mg/L) in bottom waters of the Rappahannock River (A) and Chesapeake Bay (B) in July 2005; data from VIMS bottom trawl survey (Rappahannock River) and ChesMMAAP (Chesapeake Bay).

(a)



(b)



Summer Flounder & Hypoxia

	% commitment	Cost	VIMS Match	NOAA Match
Personnel				
PI (Fabrizio; 12%) **		5,469	5,469	
PI (Brill; 10%)				10,000
Graduate student stipend		17,100		
Fringe on PI salary (30%)		1,641	1,641	3,000
sub-total		24,210	7,110	13,000
Supplies				
Transmitters (60@\$300)		18,000		
Batteries for receivers		800		
Field supplies (anesthetics, chemicals, and misc. disposable supplies)		2,400		
Array hardware - lines, shackles, thimbles, swivels; mooring (26 @ \$450 each)		11,700		
Vessel fuel (\$80/d x 35 d)		2,800		
sub-total		35,700		
Travel				
Travel to field sites (VIMS vehicle rental and fuel)		3,000		
Dorm rental - graduate student (17 wks@\$50/wk)		850		
Dorm rental - assistant (3 wks@\$50/wk)		150		
Per diems - graduate student (17 wks@\$50/wk)		850		
Per diems - assistant (3 wks@\$50/wk)		150		
sub-total		5,000		
Equipment				
Receivers (3@\$1100)		3,300		
sub-total		3,300		
Vessel Rental				
Vessel rental (35 days each year)		3,500		
sub-total		3,500		
Subcontract (Bushnell; 1 mon + benefits)		9,427		
Facilities & Administrative Costs (25%)		18,584	18,068	
Total		99,721	25,178	13,000

Facilities and Administrative Costs:

F&A costs limited to 25% for funds provided by VMRC.

Institutional approved rate is 45%. The remaining costs are contributed as part of VIMS match for this project.

** In the event that more than 1 proposal is funded, we will adjust the requested amount.

